Influence of various quantitative trait loci (QTL) mapping methods on the mapping accuracy under varying heritability levels

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ABSTRACT. The study of quantitative trait effects is of great significance for molecular marker-assisted breeding. The accuracy of quantitative trait loci (QTL) mapping is the key factor affecting marker-assisted breeding, and is extremely significant. The effect of different heritability rates (10, 30, 50, 70, and 90%) on the accuracy of QTL mapping of five recombinant inbred lines (RILs) were analyzed via computer simulation. RILs display additive and epistatic genetic effects. The QTLs were analyzed using four different mapping procedures: multiple QTL model (MQM), composite interval mapping (CIM), multiple interval mapping (MIMR), and inclusive composite interval mapping (ICIM). The results revealed an increase in the QTL mapping accuracy and QTL detection power, and a decrease in the QTL interval range with the increase in heritability; conversely, an irregular number of false positive QTLs were generated. CIM and MQM only screen the additive and dominant effects; MIMR and ICIM screen the additive, dominant, and epistatic effects. The highest QTL detection power obtained using MQM and CIM was only 75%, while MIMR and ICIM showed a detection power of 100%. At heritability rates of more than 50 and less than 10%, the detection powers of the MIMR and ICIM procedures were >95

and <35%, respectively. QTL mapping has no significance at heritability rates <10%. The results of this study suggest that QTL mapping has significance at a heritability rate >30% (at least >10%) for practical marker-assisted breeding.

Key words: QTL mapping; Heritability; Multiple procedures; Accuracy; Detection power; Computer simulation

INTRODUCTION

The late nineteenth century was witness to a rapid development in genetic linkage maps, QTL mapping methods, and related research on molecular genetics, accompanying the advancement in molecular marker technology. This has provided breeders with a powerful tool to study the genetic mechanism of quantitative traits of DNA at a molecular level. The mapping of quantitative trait loci (QTL) in plants and animals has become a worldwide phenomenon. A previous study has reported the mapping of 1287, 630, and 657 QTLs in pigs, cattle, and chicken (among animals), respectively, up to August, 2006 (Hu et al., 2007). Among plants, over 2000 QTLs have been reported (mapped) in soybean up to 2010 (Su et al., 2010a). QTLs can be used with high accuracy in marker-assisted breeding or map-based cloning; however, false-positive QTLs cannot be utilized for these applications. Although QTL mapping has developed quickly over the past few years, a large number of these mapped QTLs cannot be used in marker-assisted breeding because of their low accuracy and dependability. The results of QTL mapping performed repeatedly (several times) have always been different (Beavis, 1994, 1998). Based on its accuracy, a genetic linkage map could be used for further marker-assisted breeding. The accuracy of QTL mapping is dependent on the detection power, the identified QTL range, and accuracy of the genetic parameters. Effective strategies must be developed and analyzed to increase the accuracy and dependability of QTL mapping; this is especially of significance in the case of marker-assisted breeding in plants.

Several QTL mapping methods, such as interval mapping (IM), composite interval mapping (CIM), and multiple interval mapping (MIM), have been developed and improved gradually over time. Mapmaker/QTL is the earliest QTL analyzing software, reported for the first time by Lander and Bostein (1989). Mapmaker/QTL is based on the IM model. Since then, other QTL mapping software, such as QTL Cartographer (Basten et al., 1994), PLABQTL (Utz and Melchinger, 1996), Map Manager (Manly et al., 2001), QGene (Nelson, 1997), MapQTL (van Ooijen and Maliepaard, 1996), PGRI (Lu and Liu, 1995), QTLMAPPER (Wang et al., 2003), QTLSTA, IciMapping (Li et al., 2007), and QTLnetwork (Yang et al., 2007, 2008) have been developed and reported. A number of QTL mapping software or software packages can be downloaded freely from a website created by the University of Wisconsin-Madison (http://www.stat.wisc.edu/~yandell/qtl/software/) (Su et al., 2013). The composite interval mapping (CIM) and multiple interval mapping (MIM) functions of the WinQTL Cartographer v.2.5 (Wang et al., 2007) software program, the multiple-QTL model (MQM) of the MapQTL v.5.0 (van Ooijen et al., 2004) program, and the inclusive composite interval mapping (ICIM) model of the IciMapping v.2.0 software platform (Li et al., 2007) are four types of QTL mapping models that have found worldwide applicability.

The statistical algorithms and genetic models of the six mapping procedures are different (Utz and Melchinger, 1996; van Ooijen and Maliepaard, 1996; Kao et al., 1999; Wang, 2009). The CIM and MQM models only estimate the additive and dominant characteristics, while MIM and ICIM can be used to estimate the additive, dominant, and epistasis characteristics of the genetic models. In a practical experiment where the genetic model of the data is unknown, a multiple model

mapping strategy must be utilized (Su et al., 2013).

The heritability and marker density of the linkage map are two main factors that significantly affect the QTL mapping accuracy. Over the past few years, several researchers have attempted to analyze the QTL mapping accuracy, in order to increase the efficiency of marker-assisted breeding. The results obtained varied for different researchers because of the differences in the simulation methods, segregation population, and QTL mapping procedures used. A majority of the previous studies on QTL mapping accuracy consider only the additive and dominant effects, and not the epistatic effects.

In this study, five RIL populations with different levels of heritability (10, 30, 50, 70, and 90%) were subjected to computerized simulation and (subsequently) QTL analysis. The RIL populations displayed the same genetic model ($y = \mu + a_1 + a_2 + aa_{12} + a_3 + e$). The influence of the different levels of heritability on the accuracy of QTL mapping was analyzed in this study. The results of this study could serve as a theoretical and practical basis for future marker-assisted breeding in plants.

MATERIAL AND METHODS

Genetic model setting and analysis index

Five sets of RIL data on the additive and interactive functions between genes in a genetic model were produced (simulated 120 times) based on the differences in the heritability level. The five sets of RIL data, named RIL-1 ~ RIL-5, were completed by computer simulation, and utilized for further QTL detection. A sample size of 250 family lines was set for each simulated RIL. Three QTLs located within three linkage groups, with the genetic model "$y = \mu + a_1 + a_2 + aa_{12} + a_3 + e$", were set up. The letter y indicates the phenotypic value of quantitative characters, while the letters μ and a represent the general average number and mean additive effects, respectively. The combined aa indicates the interaction between the genes (epistatic effect), while the letter e represents the error that complies with the normal distribution "$e \sim N\,(0, \sigma_e^2)$".

The analysis index calculated for all 120 simulations considered the QTL detection power, number of false-positive QTLs, variation range of the effects of QTLs, accuracy of the QTL effects, coefficient of variation (CV) of the QTL effects, and the variation range of QTL position. The accuracy of the QTL effects was calculated using the following equation "1-|average value-set value| / set value". The QTLs identified within 10 cM (centimorgan) were considered to be identical to the QTL analyzed in this study.

RIL simulation

Marker genotype and QTL simulation

The computer simulation methodology reported by He et al. (2001) was utilized as the reference for this study. This method assumed the presence of two adjacent markers, M_k and M_{k+1}, on a chromosome with a recombination frequency r. The genotypes of the two markers (M_k and M_{k+1}) were recorded to be x_k and x_{k+1}. The values assigned to x_k and x_{k+1} (0 and 1, respectively) indicate that the DNA of the two loci arise from two homozygous parents (P1 and P2, respectively). While the value of x_k was confirmed to be "1", the genotype of x_{k+1} was inferred and confirmed from the known genotype of x_k. The presence of n_1 individuals with the genotype x_k (with a value of "1") was assumed. A total of n_1 uniformly distributed random numbers (between 0 and 1) were generated continuously, and recorded as "*rnd*". The value of x_{k+1} was determined to be "1", when

$rnd \leq (1-r)^2$. The value of x_{k+1} was determined to be "0" when $rnd > (1-r)^2$. The value of x_{k+2}, x_{k+3}, and so on, were inferred and confirmed based on the known value of x_{k+1}. The value of x_k was confirmed to be "0"; the values of x_{k+1}, x_{k+2}, x_{k+3}, and so on were inferred based on an assumed x_k value of "1".

Confirmation of the marker genotypes at the starting position of the simulated linkage group

The x_k value can be determined as 0 or 1 based on a probability of 0.50. A total of n random numbers were generated continuously between 0 and 1. The value of x_k was calculated to be 0 and 1 when the rnd values were ≤ 0.5 or > 0.50, respectively. The same method was applied to confirm the QTL marker genotype; this value was recorded as x_Q. Each simulated RIL generated a total of three linkage groups (Lg1, Lg2, and Lg3) containing 20 markers; these markers were used for further QTL analyses. Different recombination frequencies were set up between the two adjacent markers in the different linkage groups (0.06 in Lg1, 0.08 in Lg2, and 0.10 in Lg3). Three related QTLs (Q_{set1}, Q_{set2}, and Q_{set3}) were simulated according to the different genetic models of the simulated RILs. Q_{set1} was set up to be located at the marker 12 ~ marker 13 (M12 ~ M13) interval in linkage group 1 (Lg1). On the other hand, Q_{set2} and Q_{set3} were set up to be located at the M12 ~ M13 and M6 ~ M7 intervals in Lg2 and Lg3, respectively.

Confirmation of the phenotypic value of the quantity trait

The phenotypic values were set up according to the genetic model "$y = \mu + a_1 + a_2 + aa_{12} + a_3 + e$". The genetic parameters in the model were set up as follows: the genetic effect of a_1, a_2, a_3, and aa_{12} were -3, -2.5, -1.5, and 2, respectively. Different heritability levels (10, 30, 50, 70, and 90%) were set up for the five sets of simulated RILs.

QTL mapping procedures

Four types of QTL mapping procedures were utilized for further QTL analysis of the simulated data. These were the composite interval mapping (CIM) and regression forward selection of multiple interval mapping (MIMR) models provided in the WinQTL Cartographer v.2.5 software package, inclusive composite interval mapping (ICIM) function of the IciMapping (v.2.0) package, and the multiple-QTL model (MQM) provided by the MapQTL v.5.0 software platform. The data was analyzed up to one thousand permutations to determine the threshold logarithm of Odd's ratio (LOD) values.

RESULTS

Coincidence analysis between the actual and preset values when analyzed using different procedures

Different mapping procedures were applied to analyze the effects of QTL on five sets of RIL data (repeated 120 times). Several parameters, including the average, accuracy, minimum value, maximum value, and coefficient of variation (CV), were obtained by calculating and analyzing the 120 values obtained from the 120 simulations. The related results of all statistical analyses conducted on the QTL mapping power, the range of variation in the QTL position, and the numbers of false positive QTLs, are summarized in Table 1.

Table 1. Estimates of genetic parameters using simulation data of five sets of recombinant inbred line (RILs) subjected to four different quantitative trait loci (QTL) mapping procedures: multiple QTL model (MQM), composite interval mapping (CIM), inclusive CIM (ICIM), and multiple interval mapping (MIMR)).

Procedure	Effect	True value	Average	Accuracy	Minimum	Maximum	CV	RanQTL (cM)	Power (%)	False QTL
MQM, 120 times of simulation										
10%	a1	-3.00	-3.62	0.28	-5.16	-2.98	0.130	24.59	20	-
	a2	-2.50	-3.78	0.49	-5.00	-2.94	0.140	39.08		-
	aa12	2.00	-	-	-	-	-	-		-
	a3	-1.50	-3.58	0.28	-4.56	-3.12	0.148	24.20		-
30%	a1	-3.00	-2.84	0.95	-4.15	-1.78	0.160	11.46	51	-
	a2	-2.50	-2.46	0.99	-3.38	-1.77	0.149	12.42		-
	aa12	2.00	-	-	-	-	-	-		-
	a3	-1.50	-2.11	0.60	-2.63	-1.79	0.126	32.45		-
50%	a1	-3.00	-2.75	0.92	-3.51	-1.89	0.106	0.34	58	-
	a2	-2.50	-2.39	0.96	-3.18	-1.76	0.121	0.00		-
	aa12	2.00	-	-	-	-	-	-		-
	a3	-1.50	-1.63	0.91	-2.12	-1.33	0.115	38.9		-
70%	a1	-3.00	-2.81	0.94	-3.26	-2.14	0.070	0.00	65	-
	a2	-2.50	-2.37	0.95	-2.97	-1.86	0.081	0.00		-
	aa12	2.00	-	-	-	-	-	-		-
	a3	-1.50	-1.33	0.89	-1.75	-1.11	0.108	21.13		-
90%	a1	-3.00	-2.79	0.93	-3.04	-2.55	0.034	0.00	74	-
	a2	-2.50	-2.36	0.97	-2.58	-2.07	0.044	0.00		-
	aa12	2.00	-	-	-	-	-	-		-
	a3	-1.50	-1.19	0.79	-1.37	-0.97	0.074	0.39		-
CIM, 120 times of simulation										
10%	a1	-3.0	-4.11	0.63	-5.80	-2.88	0.208	28.52	27.1	-
	a2	-2.5	-3.98	0.41	-6.55	-2.93	0.182	47.15		-
	aa12	2.0	-	-	-	-	-	-		-
	a3	-1.5	-3.96	0.39	-5.56	-2.99	0.172	29.95		-
30%	a1	-3.0	-2.98	0.99	-4.50	-1.75	0.191	12.46	62.3	-
	a2	-2.5	-2.59	0.96	-3.70	-1.55	0.170	11.42		-
	aa12	2.0	-	-	-	-	-	-		-
	a3	-1.5	-2.12	0.58	-3.31	-1.53	0.218	33.45		-
50%	a1	-3.0	-2.77	0.92	-4.00	-1.38	0.145	3.87	72	-
	a2	-2.5	-2.56	0.98	-3.46	-1.75	0.131	1.87		-
	aa12	2.0	-	-	-	-	-	-		-
	a3	-1.5	-1.65	0.90	-2.83	-1.12	0.218	31.56		-
70%	a1	-3.0	-2.83	0.94	-3.41	-2.12	0.081	0.34	75	-
	a2	-2.5	-2.52	0.99	-3.33	-2.07	0.085	0		-
	aa12	2.0	-	-	-	-	-	-		-
	a3	-1.5	-1.51	0.99	-2.09	-1.04	0.148	9.99		-
90%	a1	-3.0	-2.79	0.93	-3.14	-2.52	0.040	0.34	75	-
	a2	-2.5	-2.49	1.00	-2.80	-2.17	0.049	0		-
	aa12	2.0	-	-	-	-	-	-		-
	a3	-1.5	-1.48	0.99	-1.82	-1.13	0.088	1.39		-
ICIM, 120 times of simulation										
10%	a1	-3.00	-3.89	0.70	-6.35	-2.72	0.198	30.00	26	20 (A) +2 (AA)
	a2	-2.50	-3.80	0.48	-5.43	-2.75	0.151	45.00		
	aa12	2.00	5.11	0.34	4.28	6.07	0.116	-		
	a3	-1.50	-3.71	0.32	-4.34	-3.10	0.092	33.00		
30%	a1	-3.00	-2.90	0.97	-4.95	-1.73	0.198	1.00	70	18 (A) +3 (AA)
	a2	-2.50	-2.59	0.97	3.84	-1.62	0.166	0.00		
	aa12	2.00	2.55	0.73	2.01	3.27	0.119	-		
	a3	-1.50	-2.03	0.64	-3.23	-1.57	0.162	35.00		
50%	a1	-3.00	-2.9	0.97	-4.01	-1.60	0.140	1.00	95	31 (A) +5 (AA)
	a2	-2.50	-2.54	0.99	-3.32	-1.91	0.122	1.00		
	aa12	2.00	1.91	0.96	1.37	2.72	0.152	-		
	a3	-1.50	-1.64	0.91	-2.55	-1.10	0.178	40.00		
70%	a1	-3.00	-2.94	0.98	-3.60	-2.38	0.088	1.00	98	27 (A) +5 (AA)
	a2	-2.50	-2.55	0.98	-3.36	-2.1	0.083	0.00		
	aa12	2.00	1.89	0.95	1.44	2.44	0.108	-		
	a3	-1.50	-1.55	0.97	-2.00	-0.98	0.148	0.00		

Table 1. Continued.

Procedure	Effect	True value	Average	Accuracy	Minimum	Maximum	CV	RanQTL (cM)	Power (%)	False QTL
90%	a1	-3.00	-2.89	0.96	-2.52	-3.49	0.069	0.00	100	23 (A) +7 (AA)
	a2	-2.50	-2.50	1.00	-2.79	-2.21	0.045	0.00		
	aa12	2.00	1.92	0.96	1.49	2.36	0.069	-		
	a3	-1.50	-1.54	0.97	-1.80	-1.16	0.074	0.00		
MIMR, 120 times of simulation										
10%	a1	-3.00	-4.26	0.58	-12.02	-2.91	0.327	29.52	32.1	16 (A) +1 (AA)
	a2	-2.50	-4.10	0.36	-10.40	-2.93	0.277	44.67		
	aa12	2.00	3.73	0.13	3.27	4.25	0.098	-		
	a3	-1.50	-4.61	0.52	-15.3	-2.99	0.556	28.16		
30%	a1	-3.00	-3.15	0.95	-7.73	-1.97	0.236	9.00	85.4	12 (A) +1 (AA)
	a2	-2.50	-2.63	0.95	-4.00	-1.63	0.166	5.00		
	aa12	2.00	2.10	0.95	1.53	2.97	0.166	-		
	a3	-1.50	-2.07	0.62	-4.22	-1.46	0.207	5.51		
50%	a1	-3.00	-3.00	1.00	-4.01	-1.89	0.136	0.43	95.6	23 (A)+5 (AA)
	a2	-2.50	-2.62	0.95	-3.71	-1.77	0.131	1.00		
	aa12	2.00	1.92	0.96	1.19	2.73	0.173	-		
	a3	-1.50	-1.64	0.90	-2.38	-1.07	0.203	35.45		
70%	a1	-3.00	-3.06	0.98	-3.93	-2.56	0.073	0.09	100	13 (A) +1 (AA)
	a2	-2.50	-2.57	0.97	-3.37	-2.01	0.089	0.09		
	aa12	2.00	1.92	0.96	1.30	2.46	0.106	-		
	a3	-1.50	-1.55	0.97	-2.02	-1.05	0.143	1.39		
90%	a1	-3.00	-2.98	0.99	-3.29	-2.69	0.040	0.09	100	14 (A) +2 (AA)
	a2	-2.50	-2.57	0.97	-5.46	-2.24	0.113	0.09		
	aa12	2.00	1.94	0.97	1.59	2.21	0.056	-		
	a3	-1.50	-1.56	0.96	-1.86	-1.30	0.065	0.48		

Accuracy is determined as (1-|average-true value| / true value); CV, coefficient of variation; A, additive QTL; AA, epistatic QTL pair; RanQTL, the range of QTL location.

QTL detection power

The CIM and MQM procedures could not detect the epistatic effect. The detection power of CIM and MQM was observed to be much lower than that of MIMR and ICIM; however, the heritability level was the same in all four procedures. The detection power increased with the increase in the heritability levels; the progressive increase in heritability levels from 10 to 90% resulted in a corresponding increase in the detection power of MQM from 20 to 74%, of CIM from 27.1 to 75%, of ICIM from 26 to 100%, and that of MIMR from 32.1 to 100%. The highest detection power of the MQM and CIM procedures (which can only estimate the additive and dominant effects) was 75% despite the increase in heritability. The highest detection power of MIMR and ICIM (which estimates the additive, dominant, and epistatic effects), on the other hand, was 100%. Unlike MQM and CIM, the detection power of MIMR and ICIM could be increased up to 100% by increasing the heritability level.

Variation range of QTL position

The QTLs detected within 10 cM were considered to be the same as the QTL analyzed in this study. The QTL position of the five sets of RILs, detected using different procedures, varied from 0 to 45 cM. The variation range of the QTL position showed a gradual decrease with the progressive increase in heritability levels from 10 to 90%; that is, the variation range of the QTL position was at its maximum at a heritability of 10%. The variation ranges of the Q_{set1}, Q_{set2}, and Q_{set3} positions as determined by the four different QTL mapping procedures, MQM, CIM, ICIM, and MIMR, at a heritability level of 10% were 24.59, 28.52, 30.00, and 29.52 cM, 39.08, 47.15, 45.00,

and 44.67cM, and 24.20, 29.95, 33.00, and 28.16 cM, respectively. The minimum variation range of the QTL position was observed at a heritability of 90%. The variation ranges of the Q_{set1}, Q_{set2}, and Q_{set3} positions as determined by the MQM, CIM, ICIM, and MIMR mapping procedures, were 0.00, 0.34, 0.00, and 0.09 cM, 0.00, 0.00, 0.00, and 0.09 cM, and 0.39, 1.39, 0.00, and 0.48 cM, respectively.

Conformity analysis between the actual and preset values

The actual values calculated using the different procedures and the preset values were consistent unless the mapping procedure was not fit for the genetic model data; for example, the actual value of epistasis between Q_{set1} and Q_{set2} (aa_{12}) could not be detected using the CIM and MQM procedures (which cannot determine the epistasis effects; Table 1). The genetic effects, accuracy, and CV of the same QTL fluctuated to a certain extent when subjected to 120 simulations. The accuracy of a_1, a_2, and a_3 in RIL-1, using the MQM, CIM, ICIM, and MIMR QTR mapping procedures, were 0.28, 0.30, 0.70, and 0.58, 0.49, 0.41, 0.48, and 0.36, and 0.28, 0.39, 0.32, and 0.52, respectively, in RIL-1. The accuracy of aa_{12}, as determined by ICIM and MIMR, was 0.34 and 0.13, respectively. The highest accuracy (0.70) of the actual value of a_1 was obtained using ICIM. The least variation range of the actual value of a_1 was obtained using the MQM procedure (2.18), followed by CIM (2.92). The highest accuracy of 0.49 was obtained for the actual value of a_2, when evaluated with MQM. The least variation range was obtained when the actual value of a_2 was determined using MQM (2.06) and ICIM (2.68). On the other hand, the actual value of a_3 was calculated with highest accuracy (0.52) using the MIMR procedure. The least variation range in the actual value of a_3 was obtained when determined using the MIMR and MQM procedures (1.24 and 1.44, respectively). The accuracy of a_1, a_2, and a_3, as determined by the MQM, CIM, ICIM, and MIMR QTL mapping procedures, were 0.95, 0.99, 0.97, and 0.95, 0.99, 0.96, 0.97, and 0.95, and 0.60, 0.58, 0.64, and 0.62, respectively, in RIL-2. ICIM and MIMR effected accuracies of 0.73 and 0.95 to aa_{12}, respectively. The actual values of a_1, a_2, and a_3 were determined with highest accuracy (0.99, 0.99, and 0.64) using the CIM, MQM, and ICIM models, respectively. The least variation ranges of the actual values of a_1, a_2, and a_3 were obtained using MQM and CIM (2.37 and 2.75), MQM and CIM (1.61 and 2.15), and MQM and ICIM (0.84 and 1.66), respectively. The accuracies of a_1, a_2, and a_3 in RIL-3 were determined by the MQM, CIM, ICIM, and MIMR mapping methods to be 0.92, 0.92, 0.97, and 1.00, 0.96, 0.98, 0.99, and 0.95, and 0.91, 0.90, 0.91, and 0.90, respectively. The accuracy of aa_{12} was determined to be 0.96 by both ICIM and MIMR. The actual values of a_1, a_2, and a_3 were determined with highest accuracy (1.00, 0.99, and 0.91) using the MIMR, ICIM, and both MQM and ICIM procedures, respectively. The least variation ranges of the actual values of a_1, a_2, and a_3 were obtained using MQM and MIMR (1.62 and 2.12), ICIM and MQM (1.41 and 1.42), and MQM and MIMR (0.79 and 1.31), respectively. The MQM, CIM, ICIM, and MIMR models generated a_1, a_2, and a_3 values in RIL-4 with accuracies of 0.94, 0.94, 0.98, and 0.98, 0.95, 0.99, 0.98, and 0.97, and 0.89, 0.99, 0.97, and 0.96, respectively, while ICIM and MIMR generated aa_{12} values with accuracies of 0.95 and 0.96, respectively. The actual values of a_1, a_2, and a_3 were determined with highest accuracy (0.98, 0.99, and 0.99) using the ICIM and MIMR, CIM, and CIM procedures, respectively. The least variation ranges of the actual values of a_1, a_2, and a_3 were obtained using MQM and ICIM (1.12 and 2.12), MQM (1.11), and MQM and MIMR (0.64 and 0.97), respectively. The MQM, CIM, ICIM, and MIMR QTL mapping models generated a_1, a_2, and a_3 values in RIL-5 with accuracies of 0.93, 0.93, 0.96, and 0.99, respectively, 0.97, 1.00, 1.00, and 0.97, respectively, and 0.79, 0.99, 0.97, and 0.96, respectively, while ICIM and MIMR generated aa_{12} values with accuracies of 0.97 and 0.96, respectively. The actual values of a_1, a_2,

and a_3 were determined with highest accuracy (0.99, 1.00, and 0.99) using the MIMR, CIM and ICIM, and CIM procedures, respectively. The least variation ranges of the actual values of a_1, a_2, and a_3 were obtained using the MQM and MIMR (0.49 and 0.60), MQM and ICIM (0.51 and 0.58), and MQM and MIMR (0.40 and 0.56) models, respectively.

Analysis of false-positive QTLs

The counting of false positive QTLs screened by MQM and CIM is of no significance, as these two QTL mapping procedures cannot detect the epistatic effects. Therefore, only the false positive QTL numbers generated by ICIM and MIMR were counted; the statistical results are summarized in Table 1.

The number of false positive QTLs detected by ICIM and MIMR were different when the same RIL data was simulated 120 times. The ICIM and MIMR procedures detected 20 and 16 additive and 2 and 1 pair(s) of epistatic false-positive QTLs, respectively, in RIL-1. ICIM generated 18 additive and 3 pairs of epistatic false-positive QTLs, while MIMR detected 12 additive and 1 pair of epistatic false-positive QTLs in RIL-2. On the other hand, the ICIM and MIMR procedures generated 31 and 23 additive false-positive QTLs and 5 pairs each of epistatic false-positive QTLs, respectively, in RIL-3. ICIM detected 27 additive and 5 pairs of epistatic false-positive QTLs and MIMR generated 13 additive and 1 pair of epistatic false-positive QTL in RIL-4. Finally, the ICIM and MIMR procedures detected 23 and 14 additive and 7 and 2 pairs of epistatic false-positive QTLs, respectively, in RIL-5.

In summary, the detection power of QTL was observed to increase with the increase in heritability level. The highest detection power of the MQM and CIM procedures (which cannot detect the epistatic effects of five RILs) was 75%, while MIMR and ICIM effected detection powers of 100%. The power of detection of the four QTL mapping procedures was less than 35% at QTL heritability rates of less than 10%; this can be classified as a small probability event. At QTL heritability rates of > 30%, the detection power of ICIM and MIMR was > 70 and 85%, respectively. On the other hand, at a QTL heritability of > 50%, the detection power of the ICIM and MIMR QTL mapping procedures was > 95% each. The number of false-positive QTLs generated in the five sets of simulated RILs using the same QTL mapping procedure remained unchanged. Overall, the number of false-positive QTLs detected by MIMR was lesser than that detected by ICIM for the same genetic model with the same heritability. The accuracy of QTL mapping was increased by adding the heritability level. The accuracy of both a_1 and a_2, as detected by the four QTL mapping procedures was > 90% at a QTL heritability rate of > 30%. The accuracy of a_1, a_2, a_3, and aa_{12}, as determined by the four QTL mapping procedures, was > 90% percent at QTL heritability rates of > 50%. The range of variation of the QTL position, on the other hand, became narrower with the addition of the QTL heritability level. The variation ranges of both the Q_{set1} and Q_{set2} positions were < 10 cM, when calculated using the by four QTL mapping procedures, at heritability rates of > 30%. Conversely, the variation in the range of Q_{set1} and Q_{set2} was < 5 cM (as calculated by the QTL mapping procedures) at a heritability rate of > 50%. The four QTL mapping procedures displayed variation ranges of < 10 cM for the Q_{set1}, Q_{set2}, and Q_{set3} positions at heritability rates > 70%. On the other hand, the MQM, ICIM, and MIMR QTL mapping procedures generated variation ranges < 5 cM for the Q_{set1}, Q_{set2}, and Q_{set3} positions.

We observed an increase in the accuracy of QTL mapping and QTL detection power, and a decrease in the variation range of the QTL position with the increase in heritability. In addition, we identified an irregular number of false-positive QTLs. The QTL mapping analysis is of no

significance at heritability rates < 10%. In a practical study, however, at least three strategies must be applied at QTL heritability rates < 30% to increase the confidence of the results of QTL mapping: use of multiple QTL mapping strategies (Su et al., 2013), the development of a secondary population based on the results of primary mapping, and the use of repetitive results obtained from experiments conducted over many years or locations. It has been suggested that QTL mapping has meaning at heritability rates > 30% (at least > 10%) in practical marker-assisted breeding.

DISCUSSION

The QTL analysis was performed using four different QTL mapping procedures (MQM, ICIM, CIM, and MIMR) to increase the persuasiveness and reproducibility of the five sets of computer simulated RIL data. Among these, MQM and CIM could only detect the additive and dominant effects, while ICIM and MIMR could be applied to estimate the additive, dominant, and epistatic effects. The obtained results, which shared some similar aspects with the results of a previous study (Su et al., 2010b and 2013), revealed the importance of selecting the appropriate QTL mapping procedure for different populations, using different genetic models. Many research groups have previously analyzed the QTL mapping accuracy. However, these studies were inadequate; for example, Xu (1994) analyzed only one QTL, linked to a single molecular marker with a heritability of 60%, in order to determine the influence of the QTL mapping accuracy based on the differences in the sample size. On the other hand, Beavis (1994, 1998) subjected only one marker interval, located in a single linkage, to computer simulation. A majority of researchers have considered only one QTL in their studies. The genetic parameters of the simulated QTL contains only the additive and dominant effects (always); very few studies have considered any epistatic effects of the QTLs. A majority of the previously conducted studies applied simple genetic statistical models, such as interval mapping or composite interval mapping. The above-mentioned shortcomings were overcome in this study by simulating three additive effects a_1, a_2, and a_3 and one epistatic effect aa_{12} of Q_{set1}, Q_{set2}, Q_{set3}, and the interaction between Q_{set1} and Q_{set2}. The influence of different heritability levels on QTL mapping accuracy was also analyzed using different procedures. The heritability, power of QTL detection, false positive rate, and accuracy of a majority of the QTLs is always between 10 and 30% in the factual QTL mapping process. The mechanism with which the change in heritability from 10 to 30% results in changes in the genetic parameters requires further analysis. The results of this study indicated that the QTL mapping accuracy was affected by the heritability level. The results of this study could lay the foundation for the application of marker-assisted breeding for some important agronomic traits in plants in the future.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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